cancer; and (d) it is an aspartic enzyme having a high homology to a cathepsin D precursor.

REMARKS

Enclosed herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a substitute Sequence Listing to be inserted into the specification as indicated above. The substitute Sequence Listing in no way introduces new matter into the specification.

Also submitted herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a disk copy of the substitute Sequence Listing. The disk copy of the substitute Sequence Listing, file "0020-4841P.ST25", is identical to the paper copy, except that it lacks formatting.

The substitute Sequence Listing includes a sequence disclosed in Figure 7 as filed that was not made part of the original Sequence Listing. The amendments to the Specification are being made to reference the sequences found in the Specification by their SEQ ID NOS. These amendments are editorial in nature and do not constitute new matter.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

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0020-4841P

Attachments: Paper and disk copy and of Sequence Listing
Copy of Notice to Comply
Copy of Version with Markings to Show Changes Made

COPY

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the abstract:

The abstract has been amended as follows:

An aspartic enzyme having a high homology with a cathepsin D precursor, which is a protein having the N-terminal amino acid sequence LVRIPLHKFT (SEQ ID NO:1) and showing a molecular weight of about 45 kDa in non-reductive SDS electrophoresis and can degrade plasma proteins, typically plasminogen, to produce plasma protein fragments having an inhibitory activity to metastasis and growth of cancer; the plasma protein fragments having an inhibitory activity to metastasis and growth of cancer which is prepared via the degradation with the above enzyme; a process for preparing the protein fragments which comprises degrading plasma proteins with the above enzyme; and a medicament for treating and preventing metastasis and growth of cancer which comprises as a major ingredient the above enzyme or the plasma protein fragments.

In the Specification

The paragraph beginning on page 9, line 15 has been amended as follows:

> Purification with various chromatographs revealed that this enzyme had a molecular weight of about 45 kDa. It was also found that an N-terminal amino acid sequence of this enzyme was initiated with LVRIPLHKFT (SEQ ID NO:1) which had a high homology to human Cathepsin D Precursor. Investigation with inhibitors revealed that this enzyme was classified into an aspartic enzyme. The present inventors designated this enzyme as "PACE4" (Plasminogen Angiostatin Converting Enzyme of pH 4) in connection with its exertion of the activity at a lower pH.

The paragraph beginning on page 11, line 5 has been amended as follows:

> Fig. 7 shows cleavage patterns with passage of time when plasminogen (SEQ ID NO:2) (Glu-1) is cleaved with PACE4.

The paragraph beginning on page 22, line 24 has been amended as follows:

> Identification of the Enzyme of the Present Invention
>
> After the purified enzyme sample was electrophoresed on SDS-PAGE, it was transferred to GVDF membrane by blotting. The blotted membrane was dyed with Amido Black, bands corresponding to 45 kDa were excised and the N-terminal amino acid sequence was read with an amino acid sequence analyzer. As a result, the band corresponding to 45 kDa had a sequence LVRIPLHKFT (SEQ ID NO:1). The determined amino acid sequence was compared with the existing amino acid data bank and was found to have homology with a precursor of human cathepsin D. When the enzyme purified by immunoblot was reacted with an anti-human cathepsin D antibody, said enzyme responded to this antibody. Thus, it is estimated that the enzyme of the present invention has a high homology with human cathepsin D.

The paragraph beginning on page 46, line 8 has been amended as follows:

> The enzyme of the present invention prepared from PC-3 culture supernatant, after being transferred to GVDF membrane (Immovilon, manufactured by Millipore) by blotting, was sequenced with an amino acid sequencer (Applied Biosystem Model 477A protein sequencer). This revealed that the said enzyme had the N-terminal amino acid sequence LVRIPLHKFT (SEQ ID NO:1), which was identical to that of Human Cathepsin D precursor as a result of homology search.

In the Claims

The claims have been amended as follows:

> (amended)2. The enzyme that produces plasma protein fragments of claim 1 which has the following properties: (a) it has a molecular weight of about 45 kDa as measured by SDS electrophoresis under non-reduced condition; (b) it has the N-terminal amino acid sequence LVRIPLHKFT (SEQ ID NO:1); (c) it degrades plasma proteins at an acidic pH range of not more than pH 5.0 to produce plasma protein fragments having an inhibitory activity to metastasis and growth of cancer; and (d) it is an aspartic enzyme having a high homology to a cathepsin D precursor.